Nutrient removal and nitrogen balances in high rate algal ponds with carbon dioxide addition

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Abstract The influence of CO_2 addition to high rate algal ponds (HRAPs) on nitrogen removal was investigated using two pilot-scale HRAPs operated with different hydraulic retention times (4 and 8 days). Nitrogen balances were calculated by partitioning total nitrogen into organic and inorganic nitrogen (NH_4^+-N and NO_3^--N), and to further separate the organic nitrogen components to particulate and dissolved organic nitrogen. Particulate organic nitrogen was also separated into algae organic nitrogen and bacteria organic nitrogen to investigate nitrogen concentration flow in the HRAPs. This research shows that the proportion of algae in the algal/bacterial biomass in the longer 8-day hydraulic retention time (HRT) $HRAP_{8d}$ (55.6%) was appreciably lower than that in the shorter 4-day HRT $HRAP_{4d}$ (80.5%) when CO_2 was added during the summer. Increased bacterial biomass in the HRAPs in which the maximum pH was controlled to <8.0 by CO_2 addition stimulated bacterial nitrification, indicating that biological nitrification could be an important nitrogen conversion pathway when CO_2 added to the HRAPs. Overall nitrogen removal of ~60% in the HRAPs with CO_2 addition was mainly achieved by algae/bacteria assimilation followed by solid sedimentation in a settling unit.

Keywords CO_2 addition; high rate algal ponds (HRAP); nitrogen removal; nitrogen balance

INTRODUCTION

High rate algal ponds (HRAPs), or open raceway ponds, provide better wastewater treatment, particularly nutrient removal, than conventional wastewater stabilisation ponds (Oswald, 1988; Picot, 1991; Garcia, 2000; Craggs, 2002). Nitrogen assimilation by algal cells followed by subsequent biomass separation represents the direct nitrogen removal in the HRAPs (Garcia, 2000). Elevated daytime pH (>9.0) in the HRAPs by algae photosynthetic activity results ammonia volatilization, causing indirect nitrogen removal. However, high free ammonia concentration (>30 mg/L) in pH >9.0 (20-25°C) can significantly inhibit algae growth (Azov, 1982).Therefore, algal growth and algae photosynthetic activity significantly influence on the nitrogen removal efficiency in the HRAPs.

Algal production and wastewater nitrogen removal as algal biomass in HRAPs could be enhanced by CO_2 addition to augment daytime CO_2 availability (Azov, 1982; Heubeck, 2007; Park, 2009). Controlling pond water pH to below 8 with CO_2 addition may also prevent ammonia inhibition of algal growth (Bush, 1961; Azov, 1982). While controlling HRAP water pH to below 8 might reduce nitrogen removal by physico-chemical processes such as ammonia volatilisation occurring at pH >9, increased algal assimilation could offset the reduced overall nutrient removal. However, there is little information available in the literature on pH controlled wastewater HRAPs with CO_2 addition and, in particular, the influence on nitrogen removal.

This research aimed to investigate the influence of CO_2 addition to two pilot-scale HRAPs operated with different hydraulic retention times (4 and 8 days) on nitrogen removal and the results were compared with a pilot-scale HRAP (HRT: 8d) operated without CO_2 addition. The biomass grown on the HRAPs was separated into constituent components of algae and bacteria biomass. Nitrogen balances were also calculated by partitioning total nitrogen into organic and inorganic nitrogen

(NH_4^+-N and NO_3^--N), and to further separate the organic nitrogen components to particulate and dissolved organic nitrogen. Particulate organic nitrogen was also separated into algae assimilated organic nitrogen and bacteria assimilated organic nitrogen.

MATERIALS AND METHODS

Experimental pilot-scale HRAP system

Experiments were conducted using two identical pilot-scale single-loop raceway HRAPs treating domestic wastewater at the Ruakura Research Centre, Hamilton, New Zealand (37°47'S, 175°19'E). Each HRAP had surface area of 31.8 m^2 and total volume of 8 m^3 (depth: 0.3 m) and contained a galvanised steel paddlewheel that circulated the water around pond at a mean velocity of ~0.15 m/s. The HRAPs each received anaerobic digester effluent (~1 m^3/d) which was added at the pond bottom downstream of the paddlewheel. The influent to one of the HRAPs was diluted with 1 m^3/d of tap water (simulating recycle of treated effluent after complete algae and nutrient removal) to give hydraulic retention times of 8 and 4 days ($HRAP_{8d}$ and $HRAP_{4d}$). Effluent from the HRAPs was taken from the pond bottom upstream of the paddlewheel and flowed by gravity into the mid-depth of two 250 L algal settling cones (ASCs) in parallel to separate the algal biomass. The hydraulic retention time for the ASCs following the $HRAP_{8d}$ and $HRAP_{4d}$ were 12 (ASC_{12h}) and 6 (ASC_{6h}) hours respectively. Further details of the HRAP specifications and operation were previously described in Park & Craggs (2009).

CO_2 addition

The maximum pH of the HRAPs was maintained below 8 through pH controlled CO_2 addition. The CO_2 addition system consisted of a CO_2 gas cylinder (BOC Gas Ltd, NZ), a CO_2 gas regulator, a gas flow metre (0-12 L/min range), a solenoid valve, and gas diffusers. Pond water pH was measured every five seconds with a pH probe and when the pH exceeded the pH 8 set point, the controller opened the solenoid valve and bubbled CO_2 into the ponds (2 L/min) through two CO_2 gas diffusers placed on the pond bottom in turbulent zones (one just before the paddlewheel and the other before the downstream pond corner). When the pond water pH reduced to pH 7.8 the controller closed the solenoid valve halting CO_2 addition. The pH probes were calibrated 1-2 times a week with standard pH solutions (pH 7 and 10).

Monitoring

Pond water physical properties (pH, DO and temperature) were continually measured using a DataSonde® 4a (Hydrolab, HACH Environment, USA) and data was logged at 15 minute intervals using a datalogger (CR10X, Campbell Scientific Inc, UT, USA) that downloaded daily through a wireless modem. The Datasonde® pH and DO probes were calibrated every week following manufacturer's procedures. Daily sunlight radiation was also downloaded from NIWA climate database (Ruakura AgResearch/NIWA weather station, Hamilton, New Zealand (37°47'S, 175°19'E). Weekly samples of effluent from the digester (influent), HRAPs and ASCs were taken using composite autosamplers, sampling at an hourly intervals over 24 hours. These samples were then analysed using standard methods (APHA, 2000) for the following parameters: total suspended solids (TSS), volatile suspended solids (VSS), chlorophyll-*a* (Chl-*a*), TKN, Dissolved TKN (DKN), ammoniacal-N (NH_4^+-N), nitrate-N (NO_3^--N).

Determination of algae biomass and nitrogen balances in the HRAPs

The proportion of algal biomass in the HRAPs was estimated from the chlorophyll-*a* concentration using the following equation (Raschke, 1993):

Equation 1: $$\text{Algae biomass (mg/L)} = \frac{\text{Chlorophyll} - a}{1.5} \times 100$$

This equation assumes that the algal biomass has constant chlorophyll-*a* content of 1.5% of the dry weight. Actual chlorophyll content of algae cells varies with algal species, cell density and growth conditions (particularly light availability) (Philipp, 1997). However, since both HRAP had similar dominant algae over the duration of the experiment (Park & Craggs, 2009), this method provided a useful relative measurement of the algal biomass within and between the two HRAPs.

The amount of Algae Organic Nitrogen (AON) was determined by assuming the following stoichiometry for algal production from ammoniacal-N and phosphate (Oswald, 1988).

$$106CO_2 + 236H_2O + 16NH_4^+ + HPO_4^{2-} \xrightarrow{\text{light,algae}} C_{106}H_{181}O_{45}N_{16}P + 118O_2 + 171H_2O + 14H^+$$

This assumes that algal biomass is made up of 52.4% carbon, 9.2% nitrogen and 1.3% phosphorus by weight. Although the nitrogen content of algal biomass can vary between 10.2% and 5.4% (Tillet, 1988), the 9.2% value is applicable in for algae grown on wastewater as the algal are not nitrogen limited. The nitrogen content of the algae biomass (AON) was derived using Equation 2.

Equation 2: $$\text{AON(mg/L)} = \text{Algae biomass (mg/L)} \times 9.2\%$$

Measured TKN consists of inorganic nitrogen of NH_4^+-N and organic nitrogen, which is further separated into particulate organic-N (PON) and dissolved organic-N (DON). The PON (calculated from the difference between TKN and DKN) was separated into algae organic nitrogen (AON, calculated from Equation 2) and Bacterial Organic Nitrogen (BON, calculated from the difference between PON and AON). The DON was derived from the difference between DKN and NH_4^+-N. The relationship of TKN, PON, AON, BON, DON, and NH_4^+-N are shown Equation 3.

Equation 3: TKN = NH_4^+-N + PON (AON+BON) + DON

These measured and derived nitrogen concentrations were used to determine nitrogen balance for the inflow and outflow HRAPs.

RESULTS AND DISCUSSION

The influence of CO_2 addition on nitrogen removal in two pilot-scale HRAPs operated with different hydraulic retention times ($HRAP_{8d}$: 8 days; $HRAP_{4d}$: 4 days) was investigated over 5 months by weekly monitoring during a New Zealand summer (November 07 to March 08).

Nitrogen removal by the $HRAP_{8d}$ with CO_2 addition was also compared to that of the same pond before CO_2 addition was installed (i.e. $HRAP_{8d}$ without CO_2 addition) during October 07.

pH control and physico-chemical characteristics of HRAPs

The median DO, pH and daily flow of both HRAPs were continuously monitored over the experimental period and data are summarized in Table 1. Both HRAPs achieved supersaturated DO levels during the daytime and remained aerobic at night (minimum night-time DO saturation levels were 6.9% in $HRAP_{8d}$ and 5.3% in $HRAP_{4d}$). CO_2 addition effectively controlled maximum HRAP water pH to <8.0 in both ponds (Table 1). Both ponds had a median wastewater flow of 0.90 m^3/d and 1 m^3/d of tap water was also added to the $HRAP_{4d}$.

Table 1: DO (% saturation), pH, and daily flow in the $HRAP_{4d}$ with CO_2 addition and the $HRAP_{8d}$ with and without CO_2 addition

		Without CO_2 addition	With CO_2 addition	
		$HRAP_{8d}$	$HRAP_{8d}$	$HRAP_{4d}$
DO	Median±s.d.	48.0±78.8	53.7±78.0	55.9±59.5
(% Saturation)	Max/Min	203.3/0.4	222.4/6.9	169.1/5.3
pH	Median±s.d.	7.53/0.59	7.23±0.29	7.17±0.46
	Max/Min	9.15/6.99	7.92/6.67	7.97/6.25
Flow (m^3/d)	Median±s.d.	0.95±0.05	0.89±0.12	0.90±0.12
Tap water dilution (m^3/d)	Median±s.d.	-	-	1.0

The variations in VSS and chlorophyll-*a* concentrations in both HRAPs are compared with the variations in solar radiation, pond water temperature in Figure 1. Solar radiation increased from 20.7 MJ/m^2 in November 2007 to 24.4 MJ/m^2 in January 2008 and then declined to 16.4 MJ/m^2 by March 2008. During the same period the water temperature of both ponds increased from 16.8 to 23.5°C and then declined to 20.1°C. Peak algae/bacteria biomass concentrations (measured as VSS) and chlorophyll-*a* concentration were measured on the 13th February 2008 (1060 mg VSS/L and 6.5 mg Chl-*a*/L in the $HRAP_{8d}$). Maximum areal biomass productivities (24.7 g VSS /m^2/d in $HRAP_{4d}$ and 18.5 g VSS/m^2/d in $HRAP_{8d}$) occurred in January 08 (Park & Craggs, 2009), when both solar radiation and pond water temperature were at a maximum, indicating the importance of these parameters to algal growth in the HRAPs (Oswald, 1960; Benemann, 1978; Oswald, 1988; Tillett, 1988).

The proportion of algae in the algal/bacterial biomass of the $HRAP_{8d}$ (55.6%) was appreciably lower than that of the $HRAP_{4d}$ (80.5%) (Figure 2). These results suggest that the longer 8-day hydraulic retention time in the $HRAP_{8d}$ was too long for efficient algal growth (probably as a result of self-shading) but promoted the growth of bacteria (which were unaffected by light limitation and no longer limited by high pH due to CO_2 addition (Oswald, 1988; Park, 2009)).

Nitrogen removal and nitrogen balances in the HRAPs

The median concentrations of different nitrogen species in the influent, HRAP effluent and ASC effluent are shown in Table 2. During the period when $HRAP_{8d}$ was operated without CO_2 addition (8d HRT) the influent total nitrogen concentration was 50.0 mg/L and was composed of 84% NH_4^+-N, 13.4% PON and 0.03% DON. The composition of the total nitrogen (36.7 mg/L) in the $HRAP_{8d}$

pond effluent was 29.4% NH_4^+-N, 63% PON and 7% DON, and there was very little oxidized nitrogen (mostly NO_3^--N) present (<0.01%). This suggests that a large amount of the influent nitrogen (mainly NH_4^+-N) was assimilated into algal/bacterial biomass. Using Equations 1 and 2 the amount of AON was calculated and the BON determined by subtracting the AON from the PON. Therefore, the 63% PON could be divided into 47.3% AON and 16.1% BON, indicating 3 times more algal biomass than bacterial biomass in the pond effluent.

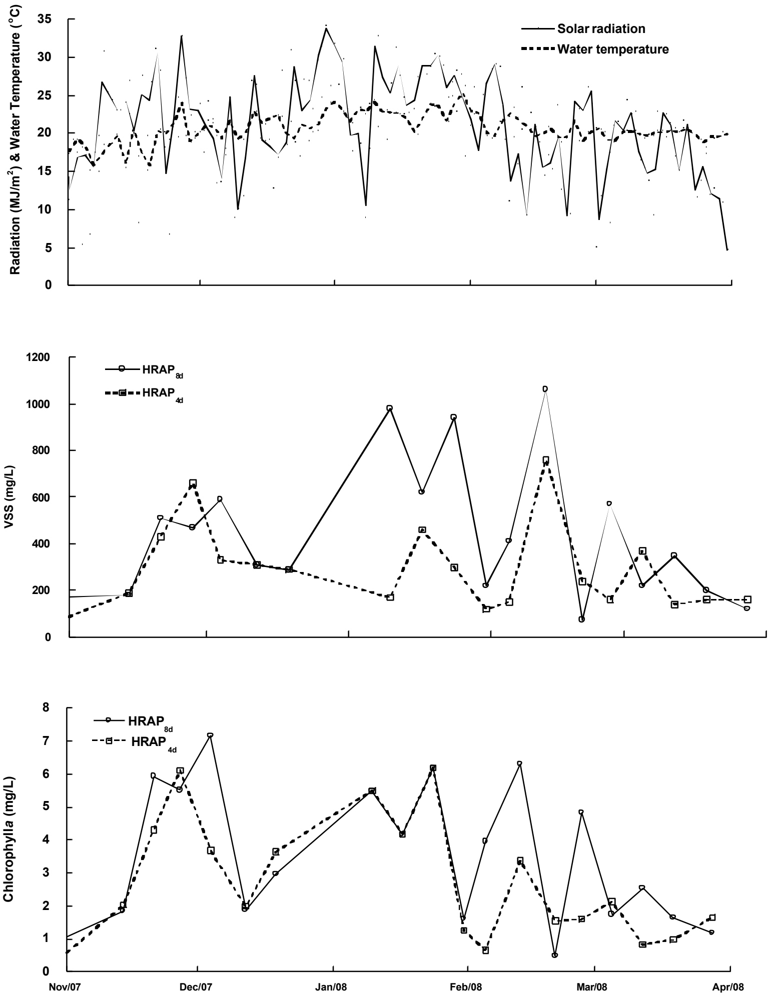


Figure 1: Variation of solar radiation and water temperature (top), biomass concentration (VSS, middle) and chlorophyll-*a* (bottom) in $HRAP_{8d}$ and $HRAP_{4d}$ with CO_2 addition.

Approximately 26% (13.3 mg/L) of the influent nitrogen could not be accounted for in the HRAP effluent and was probably lost through a combination of NH_4^+-N volatilization when the pond water pH was >9.0, denitrification of nitrate during anoxic conditions in the HRAP at night, and sedimentation of some algal/ bacterial biomass on the HRAP bottom. Garcia et al, (2000) reported that NH_4^+-N volatilization at pH >9.0 in pilot-scale HRAPs (HRT: 8d; volume: 0.47 m^3) contributed to 32% of total nitrogen removal (influent TN: 58.2 mg/L). While ammonia volatilization, dentrification or solid sedimentation in the HRAP were not measured in this study, ammonia volatilization at high pH >9 was probably the major mechanism for nitrogen loss in the $HRAP_{8d}$.

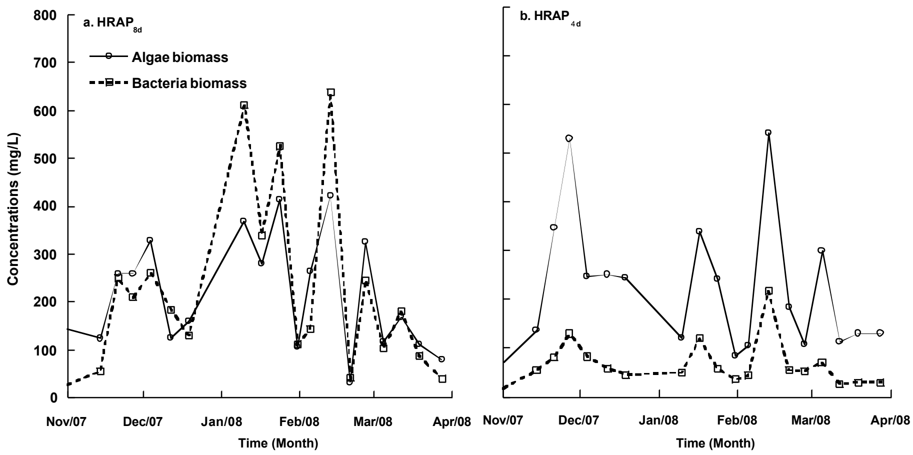


Figure 3: Algal and bacterial biomass concentrations in $HRAP_{8d}$ and $HRAP_{4d}$ with CO_2 addition during the experiment period

The higher TN concentration (64.6 mg/L) of the influent wastewater during the summer period than that during the period of operation without CO_2 addition (50.0 mg/L) was mainly due to an increased ammoniacal-N concentration which could have resulted from increased release from digestion of accumulated sludge in the anaerobic digester at the higher summer temperature. The proportion of PON (63%) in the $HRAP_{8d}$ effluent was as same as that measured in the effluent of the $HRAP_{8d}$ without CO_2 addition. However, CO_2 addition and maximum pond water pH of <8, and an 8 day HRT in the $HRAP_{8d}$ promoted bacterial growth so that there was 1.3 times more algal biomass than bacterial biomass in the $HRAP_{8d}$ effluent (BON: 29.5%; AON: 36.7%). The longer HRT and increased bacteria biomass in the $HRAP_{8d}$ promoted nitrification of NH_4^+-N to NO_3^--N ($HRAP_{8d}$ effluent NO_3^--N concentration: 9.6 mg/L). Nitrification was not observed in the $HRAP_{8d}$ without CO_2 addition when the maximum pond water pH was >9.0. This indicates that bacterial growth and nitrification may be enhanced on the HRAP with CO_2 addition at longer HRTs.

CO_2 addition to the $HRAP_{8d}$ enhanced nitrogen assimilation by algae and bacteria and reduced loss of total nitrogen from the $HRAP_{8d}$ water from 26% to 8.6%, probably indicating a reduction in ammonia volatilization with CO_2 addition and maximum pH control to <8.

Total NH_4^+-N removal of 84.5% (56.5 to 8.8 mg/L) was achieved by biomass assimilation and nitrification. The nitrogen balance in the $HRAP_{8d}$ with CO_2 addition indicates that main nitrogen removal mechanism is algae/bacteria nitrogen assimilation in the pond followed by gravity sedimentation in the ASCs (TN removal efficiency of 54.9%). Overall nitrogen balance of influent, the $HRAP_{8d}$ effluent and the ASC_{12h} effluent is described in Figure 4.

The influent nitrogen concentrations to the $HRAP_{4d}$ were halved (TN: 32.3 mg/L, TKN: 32.3 mg/L, PON: 3.0 mg/L, DON: 1.1 mg/L and NH_4^+-N: 28.3 mg/L) with tap water dilution (1 m^3/d), which was operated in parallel with the $HRAP_{8d}$ to compare nitrogen removal in a shorter retention time of 4 days. The proportion of PON (20.7 mg/L, 67%) in the $HRAP_{4d}$ was nearly as same as that of the $HRAP_{8d}$ (63%). However, appreciably higher proportion of AON (15.7 mg/L, 51%) was measured in the $HRAP_{4d}$ compared with that of $HRAP_{8d}$ (36.7%), indicating that algae nitrogen assimilation was the main nitrogen conversion pathway in the shorter HRT $HRAP_{4d}$ compared with that in the $HRAP_{8d}$. The AON to BON ratio in the $HRAP_{4d}$ was 3.2:1.Although oxidation of NH_4^+-N to NO_3^--N by nitrification also occurred in the $HRAP_{4d}$ (NO_3^--N: 2.8 mg/L), the proportion of NO_3^--N (9%)

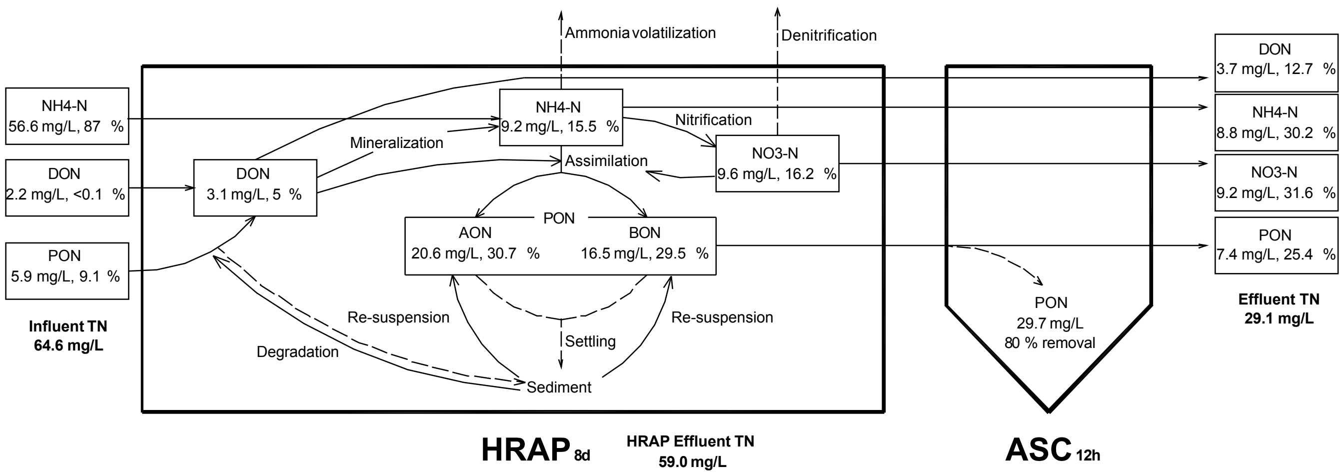
is much lower than that measured in the $HRAP_{8d}$ (16.2%).. The nitrogen removal efficiencies in the $HRAP_{4d}$ (TN removal of 59.1%, TKN removal of 67.8% and NH_4^+-N removal of 83.3%) were nearly comparable to that measured in the CO_2 added $HRAP_{8d}$. Only 5% N loss (1.5 mg/L) was measured in the $HRAP_{4d}$ containing the biomass concentration of ~280 mg VSS/L.

Enhancement of algae biomass production in HRAPs followed by efficient harvesting by a solid separation unit would be a key issue for nitrogen removal from wastewater when CO_2 added to the HRAPs.

Table 2. Nitrogen removal and nitrogen balances in the $HRAP_{8d}$ with and without CO_2 addition and in the $HRAP_{4d}$ with CO_2 addition (Note: negative removal % indicates increase compared with the influent).

	Without CO_2 addition				CO_2 addition						
		$HRAP_{8d}$	ASC_{12h}			$HRAP_{8d}$	ASC_{12h}		$HRAP_{4d}$	ASC_{6h}	
All units (g/m^3)	Influent	Effluent	Effluent	% removal	Influent	Effluent	Effluent	% removal	Effluent	Effluent	% removal
TN	50.0±9.6	36.7±7.4	32.0±8.5	36.0	64.6±10.1	59.0±11.2	29.1±12.6	54.9	30.8±15.5	13.0±9.3	59.1
TKN	50.0±9.6	36.6±7.4	31.9±8.6	36.2	64.6±10.1	49.4±10.0	19.9±10.6	69.1	28.0±16.0	10.4±6.3	67.8
PON	6.7±4.1	23.2±6.5	18.9±8.3	-182.1	5.9±3.6	37.1±13.0	7.4±12.0	-25.4	20.7±15.5	4.4±2.5	-46.7
AON	-	17.3±6.7	14.6±6.4	-	-	20.6±11.3	5.8±7.2	-	15.7±11.0	3.7±1.3	-
BON	-	5.9±0.2	4.3±1.8	-	-	16.5±1.7	1.6±4.8	-	5.0±4.5	0.7±0.7	-
DON	1.4±2.4	2.6±1.3	2.1±1.0	-50.0	2.2±2.5	3.1±1.9	3.7±3.0	-68.2	0.7±0.7	1.2±1.1	-9
NH_4^+-N	42.0±9.1	10.8±5.4	10.9±5.5	74.0	56.6±9.4	9.2±6.1	8.8±5.3	84.5	6.6±2.8	4.8±2.9	83.3
NO_3^--N	-	0.1±0.2	0.1±0.3	-	-	9.6±2.2	9.2±3.2	-	2.8±0.8	2.6±0.2	-

Figure 4: The nitrogen balances in the $HRAP_{8d}$ with CO_2 addition (Note: the dotted lines are the nitrogen removal pathway and the solid lines are the nitrogen conversation to other forms.



CONCLUSIONS

This study investigating the influence of CO_2 addition on nitrogen removal by two pilot-scale HRAPs operated with different hydraulic retention times (4 and 8 days) has shown that:

1. The proportion of algae in the algal/bacterial biomass growing in HRAP with CO_2 addition was appreciably lower in the longer 8-day HRT $HRAP_{8d}$ (55.6%) than that in the shorter 4-day HRT $HRAP_{4d}$ (80.5%).
2. Control of the HRAP water maximum pH to less than pH 8.0 with CO_2 addition stimulated nitrification particularly in the longer HRT $HRAP_{8d}$, indicating that nitrifying bacteria are inhibited by the high daytime pH in HRAP without CO_2 addition and may have been washed out of the short HRT HRAP with CO_2 addition.
3. The majority of nitrogen removal in both HRAPs with CO_2 addition was achieved by algal/bacterial assimilation followed by biomass sedimentation in the settling units.

Further research is required to determine how nitrogen removal may be improved through a combination of increased algal production and assimilation, and improved biomass removal in the solid settling units.

REFERENCES

APHA, 2000. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington.

Azov, Y., Goldman, J. C., 1982. Free Ammonia Inhibition of Algal Photosynthesis in Intensive cultures. *Applied and Environmental Microbiology*, 43(4), 735-739.

Benemann, J.R., Koopman, B.L., Weissman, J.C., Eisenberg, D.M., Oswald, W.J., 1978. An integrated system for the conversion of solar energy with sewage-grown microalgae. *Report, Contract D(0-3)-34*, U.S. Dept. of Energy, SAN-003-4-2, June 1978.

Bush, A.F., Isherwood, J. D. and Rodgi, S., 1961. Dissolved solids removal from waste water by algae. *Journal of the Sanitary Engineering Division - Proceedings of the American Society of Civil Engineers*, 87(SA3), 39-57.

Craggs, R.J., R. J. Davies - Colley, C. C. Tanner and J. P. Sukias., 2002. *Advanced Pond System: performance with High Rate Ponds of different depths and areas*, 5th international IWA specialist group conference on waste stabilisation ponds, New Zealand Water and Wastes Association, Sky City Hotel, Auckland, New Zealand.

Garcia, J., Mujeriego, R., Hernandez-Marine, M, 2000. High rate algal pond operating stategies for urban wastewater nitrogen removal. *Journal of Applied Phycology*, 12, 331-339.

Heubeck, S., Craggs, R. J., Shilton, A., 2007. Influence of CO2 scrubbing from biogas on the treatment performance of a high rate algal pond. *Water Science and Technology*, 55(11), 193.

Oswald, W.J., 1988. *Microalgal biotechnology*. Borowitzka, M.A., Borowitzka, L.J (eds). Cambridge University Press.

Oswald, W.J., Golueke, C.G., 1960. Biological transformation of solar energy. *Adv. Appl. Microbiol.*, 11, 223-242.

Park, J.B.K., Craggs, R. J. , 2009. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science and Technology*.

Philipp, M., Cuhel, R., Nyholm, N., 1997. A simple in vitro fluorescence method for biomass measurement in algal growth inhibition tests. *War. Res*, 31(10), 525-253.

Picot, B., El Halouani, H., Casellas, C., Moersidik, S., Bontoux, J., 1991. Nutrient removal by high rate pond system in a mediterranean climate (France). *Water Sci. Technol*, 23, 1535-1541.

Raschke, R.L., 1993. Diatom community response to phosphorous in the Everglades National Park USA. *Phycologia*, 32(1), 48-58.

Tillett, D.M., 1988. Ph.D Thesis: Lipid productivity and species competition in laboratory models of algae mass cultures., Georgia Institute of Technology, 316 pp.